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RESEARCH NOTE

Spread of the *Streptococcus pneumoniae* Taiwan^{19F}-14 clone among children in Greece

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ABSTRACT

Serotype 19F pneumococci were a leading cause of infections among children in Athens, Greece during 2001–2006. In total, 143 19F isolates were typed by pulsed-field gel electrophoresis (PFGE), and 38 isolates representing the main PFGE types were also characterised by multilocus sequence typing. A diversity of distinct strains belonging to sequence types 236, 1035, 274, 172 and 319 were identified, but multidrug-resistant isolates related to the Taiwan^{19F}-14 clone (ST236) constituted 76.9% of the isolates. Spread of the Taiwan^{19F}-14 clone explains, in part, the high incidence of antibiotic resistance observed among pneumococci reported recently from Athens.

Keywords Children, epidemiology, Greece, pneumococci, *Streptococcus pneumoniae*, Taiwan^{19F}-14

Original Submission: 19 April 2007; **Revised Submission:** 5 July 2007; **Accepted:** 17 July 2007

Clin Microbiol Infect 2007; **13:** 1213–1216
10.1111/j.1469-0691.2007.01837.x

Treatment of pneumococcal infections can be complex because of the dissemination of clones

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Table 1. Pneumococcal isolates from Athens, Greece, showing the source of the serotype 19F isolates included in the study

	2001–2004			2005 to April 2006		
Source	**Total**	**19F (%)**	**Studied**	**Total**	**19F (%)**	**Studied**
IPD	186	12 (6.5)	8	36	7 (19.4)	7
NIPD	641	279 (43.5)	53	133	51 (38.3)	35
Carriage	206	37 (18.0)	16	189	35 (18.5)	24
Total	1033	328 (31.8)	**77**	358	93 (26.0)	**66**

IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease.

exhibiting resistance to various antibiotics, including penicillin and macrolides [1,2]. High isolation rates of multidrug-resistant pneumococci from children in Greece have been reported. These isolates were commonly recovered from non-invasive pneumococcal disease, mainly acute otitis media, but also from healthy carriers, and often belonged to serotype 19F [3–5]. The present study investigated 143 pneumococci of serotype 19F isolated between January 2001 and April 2006 from children (aged ≤14 years) in Athens (Table 1). In the pre-vaccination period (2001–2004), serotype 19F isolates constituted a significant proportion (31.8%) of pneumococci isolated in the main paediatric hospitals of Athens (Aghia Sofia, A. & P. Kyriakou and Penteli Hospitals). The characteristics of the pre-vaccination isolates have been described previously [5]. Seventy-seven of the 328 serotype 19F isolates from this collection were available for inclusion in the present study (Table 1). Between 2005 and April 2006, 169 isolates were recovered from cases of invasive pneumococcal disease and non-invasive pneumococcal disease among children attending a post-vaccination sentinel hospital in Athens (Aghia Sofia) for the follow-up of pneumococcal infections. Of these, 58 (34.3%) isolates belonged to serotype 19F, and this serotype was also common (18.5%) among 189 isolates derived from healthy carriers during the post-vaccination period. Sixty-six isolates of serotype 19F (42 from infections and 24 from carriers) had been retained in the hospital laboratory and were included in the study (Table 1). An additional isolate (UK-GR), representing an erythromycin-resistant 19F clone isolated in 1996 [6], was also included for comparison.

Serotypes were determined using the Quellung reaction with pooled and selected factor antisera (Statens Seruminstitut, Copenhagen, Denmark). Penicillin, amoxycillin, cefotaxime and ofloxacin MICs were determined by Etests (AB Biodisk, Solna, Sweden). Susceptibility to erythromycin, clindamycin, co-trimoxazole, tetracycline, chloramphenicol and rifampicin was assessed by disk-diffusion, interpreted as recommended by the CLSI [7]. Phenotypic characterisation of macrolide resistance was also performed [8].

Pneumococci were typed by pulsed-field gel electrophoresis (PFGE), with preparation of genomic DNA and electrophoresis conditions as described previously [6]. Isolates with PFGE patterns differing by one to six bands were assigned to different subtypes within the same type [9]. Isolates representing the main PFGE types and subtypes were also characterised by multilocus sequence typing (MLST), based on sequence analysis of seven housekeeping genes, i.e., *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt* and *ddl* [10]. Sequences were determined on both DNA strands, and were compared with those available at the MLST website (http://www.mlst.net). Novel alleles and new sequence types (STs) were assigned new numbers and deposited in the *Streptococcus pneumoniae* MLST database. Clonal complexes (CCs) were defined using EBURST software.

PFGE typing of 143 pneumococci belonging to serotype 19F revealed 18 types, further divided into a total of 55 subtypes. Ten types were represented by one isolate each, while two or more isolates clustered in the remaining eight types, labelled A (six isolates; five subtypes), B (two; two), C (two; two), D (three; two), E (two; two), F (six; four), G (110 isolates, 55 of which belonged to only one of the 30 subtypes), and H (two; two) (Table 2). The 19F erythromycin-resistant clone described previously (UK-GR), derived from children in Athens in 1996 [6], also belonged to type G, but represented a distinct subtype not observed among the current isolates.

Thirty-six isolates belonging to seven PFGE types (A, C, D, E, F, G and H) and two isolates exhibiting unique PFGE patterns were further typed by MLST. Thirteen STs, distributed into seven CCs, were identified (Table 2). Five allelic profiles had not been observed previously and were assigned new ST numbers. PFGE type G isolates were either ST236 (15-16-19-15-6-20-26) (n = 25, including the UK-GR isolate from 1996), corresponding to the Taiwan^{19F}-14 clone, or were novel single-locus variants of ST236: ST 2320 (15-16-19-15-6-193-26); ST 2321 (15-16-19-15-6-20-

Table 2. Characteristics of 143 *Streptococcus pneumoniae* isolates belonging to serotype 19F

PFGE type (*n*)	Isolation period	Clinical source (*n*)	Resistance phenotype (*n*)	ST	CC
A (6)	2001–2003, 2006	IPD (2), NIPD (2), carriage (2)	Susceptible (3), PenErySxtTet (2) PenErySxtTetCli (1)	1035	162
B (2)	2005	IPD (1), carriage (1)	Susceptible (2)	ND	
C (2)	2004, 2005	NIPD (2)	Susceptible (2)	274	199
D (3)	2002, 2004	IPD (1), NIPD (2)	Susceptible (3)	172	172
E (2)	2001–2006	NIPD (1), carriage (1)	Susceptible (1), PenErySxtTetCli (1)	319	230
F (6)	2002–2005	NIPD (5), carriage (1)	Susceptible (3), PenErySxtTet (1), PenErySxtTetCli (1), PenEryTet (1)	177	177
G (110)	2001–2006	IPD (11), NIPD (67), carriage (32)	PenErySxtTet (75), PenEryTet (12), PenCtxErySxtTet (10), PenErySxt (8), PenCtxErySxtTetCli (3), PenErySxtTetCli (2)	236, 2320, 2321, 2322, 2323	271
H (2)	2004, 2006	Carriage (2)	Susceptible (1), ErySxtTet (1)	344	344
Unique types (10)	2001–2006	NIPD (9), carriage (1)	Susceptible (3), PenErySxtTet (6), ErySxtTet (1)	416, 2324	199, 177

PFGE, pulsed-field gel electrophoresis; Pen, penicillin; Ctx, cefotaxime; Ery, erythromycin; Sxt, co-trimoxazole; Tet, tetracycline; Cli, clindamycin; ND, not determined; IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease; ST, sequence type; CC, clonal complex.

226); ST 2322 (15-16-19-15-6-20-228); and ST 2323 (7-16-19-15-6-20-26). The latter ST is probably derived from an ST236 strain by recombination in the *aroE* locus. Thus, typing indicated that the majority of the multidrug-resistant serotype 19F pneumococci were indistinguishable from, or closely related to, the international clone Taiwan^{19F}-14 (CC271). Both isolates of PFGE group F belonged to ST177, corresponding to the Portugal^{19F}-21 clone (CC177). Isolates of PFGE types A, C, D, E and H were ST1035 (CC162), ST274 (CC199), ST172 (CC172), ST319 (CC230) and ST344 (CC344), respectively. Two isolates with unique PFGE patterns were ST416 (CC199) and ST2324 (a novel single-locus variant of ST177).

All Taiwan^{19F}-14-related isolates were multidrug-resistant (i.e., resistant to three or more drug classes), compared with only 15 (45.5%) of the remaining 33 pneumococci belonging to all other types (Table 2). All were penicillin-non-susceptible, exhibiting either intermediate (MICs 0.064–1 mg/L) (43.6%) or high-level (MICs ≥2 mg/L) (56.4%) resistance. Thirteen (11.8%) of the penicillin-non-susceptible type G isolates were also non-susceptible to cefotaxime (MICs 2–8 mg/L). Isolates of this cluster were all resistant to erythromycin, but only 4.5% were resistant to clindamycin. The M phenotype, i.e., resistance to erythromycin and susceptibility to clindamycin, thus predominated (95.5% of the 110 Taiwan^{19F}-14-related isolates), which is consistent with previous reports suggesting an association of this clone with *mef* genes [11,12]. Rates of resistance to co-trimoxazole and tetracycline among these isolates were 89.1% and 92.7%, respectively. All 143 isolates were susceptible to amoxycillin, chloramphenicol, ofloxacin and rifampicin.

The Taiwan^{19F}-14 clone is disseminated mostly in Asian countries, South Africa and the USA [11–14], and is isolated only infrequently in Europe [15–17]. The present study provides evidence of the persistence and spread of isolates of this clone among children in Greece, thereby accounting, to a large extent, for the high resistance rates observed recently among pneumococci in Greece [4,5]. Taiwan^{19F}-14-related isolates have circulated in this community since at least 1996, as indicated by typing of the UK-GR clone. Another ST236 strain (JJ271-19F), isolated in Greece during 1997, has been found in the MLST database, thereby providing further evidence that this ST was present before the study period, but the significance of this clone during the 1990s cannot be reliably assessed. These findings also highlight the differences among communities in the distribution of pneumococci. Indeed, in neighbouring countries, isolates related to this clone remain uncommon [18–20].

Over 75% of the Taiwan^{19F}-14-related pneumococci belonged to the same PFGE type, G. However, differences in both phenotypes and DNA fingerprints, as well as the emergence of novel STs among type G isolates, suggest an actively evolving clone. The frequent isolation of Taiwan^{19F}-14-related pneumococci among carriers, and their over-representation among cases of acute otitis media, probably reflect the high colonisation potential of this serotype. Although it is still premature to evaluate the impact of the seven-valent conjugate vaccine, these findings are in line with previous observations suggesting that 19F is the least affected of the vaccine serotypes [21]. In addition, the high population density in Athens and the extensive use of macrolides

(among the highest in Europe) [22] have probably facilitated transmission and establishment of this clone. The low frequency of isolation of the less resistant 19F isolates that are unrelated to the Taiwan^{19F}-14 clone supports the important role of selection pressure in this setting.

ACKNOWLEDGEMENTS

We thank G. Diamantopoulou for technical assistance and C. Bishop for assigning new alleles and STs. Studies on *S. pneumoniae* epidemiology in the Department of Microbiology, School of Medicine, National University of Athens, are financially supported by the Centre for Control and Prevention of Infections (KEELPNO).

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